Third Commission Directive of 27 September 1983 on the approximation of the laws of the Member States relating to methods of analysis necessary for checking the composition of cosmetic products (83/514/EEC)

ANNEX

DETERMINATION OF DICHLOROMETHANE AND 1,1,1-TRICHLOROETHANE IDENTIFICATION AND DETERMINATION OF MERCAPTOACETIC ACID IN HAIR-WAVING, HAIR-STRAIGHTENING AND DEPILATORY PRODUCTS

1. SCOPE AND FIELD OF APPLICATION

This method describes he identification and determination of mercaptoacetic acid in hair-waving, hair-straightening and depilatory products in which other reducing agents may be present.

2. DEFINITION

The mercaptoacetic acid content of the sample determined according to this method is expressed in percentage by mass of mercaptoacetic acid.

3. PRINCIPLE

Mercaptoacetic acid is identified by spot tests and by thin-layer chromatography and is determined by iodometry or gas chromatography.

4. IDENTIFICATION

4.1. ***Identification by spot tests***

4.1.1. *Reagents*

All reagents should be of analytical purity.

4.1.1.1. Lead di(acetate) papes.

4.1.1.2. Hydrochloric acid solution (one volume of concentrated hydrochloric acid plus one volume of water)

4.1.2. Procedure

4.1.2.1. *Identification of mercaptoacetic acid by means of a colour reaction with lead di(acetate)*

Place a drop of the sample to be analyzed on lead di(acetate) paper (4.1.1.1). If an intense yellow colour appears, mercaptoacetic acid is probably present.

Sensitivity: 0,5 %.

4.1.2.2. *Characterization of inorganic sulphides by the formulation of hydrogen sulphide on acidification*

Introduce, into a test tube, a few milligrams of the sample to be studied. Add 2 ml of distilled water and 1 ml of hydrochloric acid (4.1.1.2). Hydrogen sulphide, recognizable by its smell, is evolved and a black lead sulphide precipitate forms on the lead di(acetate) paper (4.1.1.1).

Sensitivity: 50 ppm.

4.1.2.3. *Characterization of sulphites by the formation of sulphur dioxide upon acidification*

Proceed as described in 4.1.2.2. Bring to the boil. The sulphur dioxide is recognizable by its smell and by its reducing properties in respect, for example, of permanganate ions.

4.2. Identification by thin-layer chromatography

4.2.1. *Reagents*

All reagents, except where otherwise stated, should be of analytical purity.

4.2.1.1. Mercaptoacetic acid (thioglycollic acid), 98 % minimum purity assayed by iodometry.

4.2.1.2. 2,2'-dithiodi(acetic acid), 99 % minimum purity assayed by iodometry.

4.2.1.3. 2-mercaptopropionic acid (thiolactic acid), 95 % minimum purity assayed by iodometry.

4.2.1.4. 3-mercaptopropionic acid, 98 % minimum purity assayed by iodometry.

4.2.1.5. 3-mercaptopropane-l,2-diol (1-thioglycerol), 98 % minimum purity assayed by iodometry.

4.2.1.6. Thin-layer plates, silica gel, ready prepared, 0,25 mm thickness.

4.2.1.7. Thin-layer plates, aluminium oxide, Merck F 254 E or equivalent.

4.2.1.8. Hydrochloric acid, concentrated, d_4^{20} = 1,19 g/ml.

4.2.1.9. Ethyl acetate.

4.2.1.10. Chloroform.

4.2.1.11. Diisopropyl ether

4.2.1.12. Carbon tetrachloride.

4.2.1.13. Acetic acid, glacial.

4.2.1.14. Potassium iodide, 1 % (m/v) solution in water.

4.2.1.15. Platinum tetrachloride, 0,1 % (m/v) solution in water.

4.2.1.16. *Eluting solvents*

4.2.1.16.1 Ethyl acetate (4.2.1.9), chloroform (4.2.1.10), diisopropyl ether (4.2.1.11), acetic acid (4.2.1.13) (20: 20: 10: 10, by volume).

4.2.1.16.2 Chloroform (4.2.1.10), acetic acid (4.2.1.13) (90: 20, by volume).

4.2.1.17. *Detection reagents*

4.2.1.17.1 Mix, immediately before use, equal volumes of solution (4.2.1.14) and solution (4.2.1.15).

4.2.1.17.2 Bromine solution 5 % (m/v):

Dissolve 5 g of bromine in 100 ml of carbon tetrachloride (4.2.1.12).

4.2.1.17.3 Fluorescein solution, 0,1 % (m/v):

Dissolve 100 mg of fluorescein in 100 ml of ethanol.

4.2.1.17.4 Hexaammonium heptamolybdate, 10 % (m/v) solution in water.

4.2.1.18. *Reference solutions*

4.2.1.18.1 Mercaptoacetic acid (4.2.1.1), 0,4 % (m/v) solution in water.

4.2.1.18.2 2,2'-dithiodi(acetic) acid (4.2.1.2), 0,4 % (m/v) solution in water.

4.2.1.18.3 2-mercaptopropionic acid (4.2.1.3), 0,4 % (m/v) solution in water.

4.2.1.18.4 3-mercaptopropionic acid (4.2.1.4), 0,4 % (m/v) solution in water.

4.2.1.18.5 3-mercaptopropane-l,2-diol (4.2.1.5), 0,4 % (m/v) solution in water.

4.2.2. *Apparatus*

Usual apparatus for thin-layer chromatography.

4.2.3. *Procedure*

4.2.3.1. *Treatment of samples*

Acidify to pH 1 with a few drops of hydrochloric acid (4.2.1.8) and filter if necessary.

In certain cases it may be advisable to dilute the sample. If so acidify it with hydrochloric acid before dilution.

4.2.3.2. *Elution*

Place on the plate 1 µl of sample solution (4.2.3.1) and one litre of each of the five reference solutions (4.2.1.18). Dry carefully in a gentle current of nitrogen and elute the plate with solvents (4.2.1.16.1 or 4.2.1.16.2). Dry the plate as quickly as possible to minimize oxidation of the thiols.

4.2.3.3. *Detection*

Spray the plate with one of the three reagents (4.2.1.17.1, 4.2.1.17.3 or 4.2.1.17.4). If the plate is sprayed with reagent (4.2.1.17.3), further treat it with bromine vapour (e.g. in a tank containing a small beaker of the reagent (4.2.1.17.2)) until the spots are visible. Detection with the spray reagent (4.2.1.17.4) will be satisfactory only if the drying time for the thin layer has not exceeded 30 minutes.

4.2.3.4. *Interpretation*

Compare the Rf values and the colour of the reference solutions with those of the standards. The mean Rf values given below as a rough guide have only a comparative value. They depend upon:

— the state of activation of the thin layer at the time of chromatographing,

— the temperature of the chromatography tank.

EXAMPLES OF RF VALUES OBTAINED ON A SILICA GEL LAYER

	Eluting solvents	
	4.2.1.16.1	**4.2.1.16.2**
Mercaptoacetic acid	0,25	0,80
2-mercaptopropionic acid	0,40	0,95
2,2'-dithiodi(acetic) acid	0,00	0,35
3-mercaptopropionic acid	0,45	0,95
3-mercaptopropane-1,2 diol	0,45	0,35

5. DETERMINATION (see NB)

The determination should always start with the iodometric procedure.

5.1. ***Iodometry***

5.1.1. *Principle*

The determination is performed by oxidation of the '-SH' group with iodine in an acid medium according to the equation:

$2\ HOOC\text{-}CH_2SH + I_2 \rightarrow (HOOC\text{-}CH_2\text{-}S)_2 + 2\ I + 2\ H^+$

5.1.2. *Reagents*

Iodine, 0,05 M standard solution.

NB: The determination of mercaptoacetic acid must be carried out on unused product from freshly opened containers in order to prevent oxidation.

5.1.3. *Apparatus*

Usual laboratory equipment.

5.1.4. *Procedure*

Accurately weigh out a quantity of between 0,5 and 1 g of the sample into a 150 ml stoppered conical flask containing 50 ml of distilled water. Add 5 ml of hydrochloric acid (4.1.1.2) (pH of solution about 0) and titrate with iodine solution (5.1.2) until a yellow colour appears. Use an indicator (e.g. starch solution or carbon tetrachloride) if desired.

5.1.5. *Calculation*

The mercaptoaceric acid content is calculated according to the formula:

$$\%\ (m/m) = \frac{92 \times n \times 100}{1000 \times 10 \times m} = \frac{0{,}92\ n}{m}$$

where:

m	=	the mass (in grams) of the test portion,
n	=	the volume of iodine solution (5.1.2) used.

5.1.6. *Remarks*

If the result, calculated as mercaptoacetic acid, is 0,1 % or more below the authorized maximum concentration, there is no point in carrying out further determinations. If the result is equal to or above the permitted maximum concentration, and the identification has revealed the presence of several reducing agents, it is necessary to carry out a gas chromatographic determination.

5.2. ***Gas chromatograyhy***

5.2.1. Principle

Mercaptoacetic acid is separated from the excipient by precipitation with cadmium di(acetate) solution. After methylation with diazomethane, prepared either *in situ* or in advance in a diethyl ether solution, the methyl derivative of the mercaptoacetic acid is measured by gas/liquid chromatography, methyl octanoate being used as the internal standard.

5.2.2. *Reagents*

All the reagents must be of analytical quality.

5.2.2.1. Mercaptoacetic acid, 98 %.

5.2.2.2. Hydrochloric acid, d_{410} = 1,19 g/ml.

5.2.2.3. Methanol.

5.2.2.4. Cadmium di(acetate) dihydrate, 10 % (m/v) solution in water.

5.2.2.5. Methyl octanoate, 2 % (m/v) solution in methanol.

5.2.2.6. Acetate buffer solution (pH 5):

Sodium acetate trihydrate, 77 g.

Acetic acid (glacial), 27,5 g.

Demineralized water to give a final volume of one litre.

5.2.2.7. Hydrochloric acid, 3 M solution in methanol (5.2.2.3), freshly prepared.

5.2.2.8. 1-methyl-3-nitro 1 -nitrosoguanidine.

5.2.2.9. Sodium hydroxide, 5 M solution.

5.2.2.10. Iodine, 0,05 M standard solution.

5.2.2.11. Diethyl ether.

5.2.2.12. *Diazomethane solution prepared from iV-methyl-AT-nitrosotoluen-4-sulfonamide (Fieser, Reagents for Organic Synthesis (Wiley), 1967)*

The solution obtained contains about 1,5 g of diazomethane in 100 ml of diethyl ether. As diazomethane is a toxic and very unstable gas, all experiments must be carried out under a powerful hood and the use of ground-glass apparatus must be avoided (there are special kits for this purpose).

5.2.3. *Apparatus*

5.2.3.1. Usual laboratory equipment.

5.2.3.2. Apparatus for the preparation of diazomethane for *in situ* methylation (see Fales, H. M., Jaouni, T. M. and Babashak, J. F., Analyt. Chem. 1973, 45, 2302).

5.2.3.3. Apparatus for the advance preparation of diazomethane (Fieser).

5.2.4. *Preparation of the sample*

Weigh accurately into a 50 ml centrifuge tube enough of the sample to give a presumed quantity of 50 to 70 mg of mercaptoacetic acid. Acidify with a few drops of hydrochloric acid (5.2.2.2) to obtain a pH of about 3.

Add 5 ml of demineralized water and 10 ml of acetate buffer solution (5.2.2.6).

Check with pH paper that the pH value is about 5. Then add 5 ml of cadmium di(acetate) solution (5.2.2.4).

Wait 10 minutes and then centrifuge for at least 15 minutes at 4 000 g. Remove the supernatant liquid which may contain an insoluble fat (in the case of cream products). This fat cannot be confused with the thiols which collects in a compact mass at the bottom of the tube. Check that no precipitation occurs when a few drops of cadmium di(acetate) solution (5.2.2.4) are added to the supernatant.

Where earlier identification revealed no reducing agents other than the thiols, check by iodometry that the thiol present in the supernatant liquid does not exceed 6 to 8 % of the initial quantity.

Introduce 10 ml of methanol (5.2.2.3) into the centrifuge tube containing the precipitate and finely disperse the precipitate with a stirring rod. Centrifuge again for at least 15 minutes at 4 000 g. Pour off the supernatant and check for the absence of thiols.

Wash the precipitate a second time by the same procedure.

Still using the same centrifuge tube, add:

— 2 ml of methyl octanoate solution (5.2.2.5),

— 5 ml of hydrochloric acid in methanol (5.2.2.7).

Completely dissolve the thiols (a little insoluble matter may persist from the excipient). This is solution ‘S’.

With an aliquot of this solution, check iodometrically that the thiols content is at least 90 % of that obtained in 5.1.

5.2.5. *Methylation*

The methylation is carried out either by *in situ* preparation (5.2.5.1) or with previously prepared diazomethane solution (5.2.5.2).

5.2.5.1. *Methylation in situ*

Into the methylation apparatus (5.2.3.2) containing 1 ml of ether (5.2.2.11) introduce 50 µl of solution ‘S’ and methylate by the method (5.2.3.2) with about 300 mg of l-methyl-3 nitro-1-nitrosoguanidine (5.2.2.8). After 15 minutes (the ether solution should be yellow to indicate excess diazomethane) transfer the sample solution to a 2 ml bottle having an airtight stopper. Place in the refrigerator overnight. Methylate two samples simultaneously.

5.2.5.2. *Methylation with the previously prepared diazomethane solution*

Introduce, into a 5 ml stoppered flask, 1 ml of diazomethane solution (5.2.2.12) then 50 µl of solution ‘S’. Leave in the refrigerator overnight.

5.2.6. *Preparation of the standard*

Prepare a standard solution of mercaptoacetic acid (5.2.2.1) of known strength containing about 60 mg of pure mercaptoacetic acid (5.2.2.1) in 2 ml.

This is solution ‘E’.

Precipitate, assay and methylate as described in 5.2.4 and 5.2.5.

5.2.7. *Gas chromatographic conditions*

5.2.7.1. *Column*

Type: stainless steel.

Length: 2 m.

Diameter: 3 mm.

5.2.7.2. *Packing*

20 % didecyl phthalate/chromosorb, WAW 80 to 100 mesh.

5.2.7.3. *Detector*

Flame ionization. A suitable sensitivity setting for the electrometer of the flame ionization detector is 8×10^{-10} A.

5.2.7.4. *Gas supplies*

Carrier gas: nitrogen.

pressure: 2,2 bar,

flow: 35 ml/min.

Auxiliary gas: hydrogen.

pressure: 1,8 bar,

flow: 15 ml/min.

Detector supplies: as specified by the makers of the apparatus.

5.2.7.5. *Temperature conditions*

Injector: 200 °C

Detector: 200 °C

Column: 90 °C

5.2.7.6. *Recorder chart speed*

5 mm/min.

5.2.7.7. *Quantity injected*

3 µl Carry out five injections.

5.2.7.8. The conditions of chromatography are given as a guide. They permit the achievement of a resolution 'R' equal to, or better than, 1,5, where:

$$R = 2\frac{d'(r_2 - r_1)}{W_1 + W_2}$$

let:

r_1 and r_2 = retention times (in minutes),
W_1 and W_2 = peak widths at half height (in millimetres),
d' = the chart speed (in millimetres per minute).

It is recommended that chromatography be terminated by regulating the tempera-ture from 90 to 150 °C at a rate of 10 °C per minute so as to eliminate substances liable to interfere with subsequent measurements.

5.2.8. *Calculations*

5.2.8.1. *Coefficient of proportionality for mercaptoacetic acid*

This is calculated with respect to methyl octanoate on the basis of a standard mixture.

If 't' represents mercaptoacetic acid:

let:

k_t = its response factor,
m'_t = its mass (in milligrams) in the mixture,
S'_t = its peak area.

If ‘c’ represents methyl octanoate:

let:

m'_c = its mass (in millegrams) in the mixture,
S'_c = its peak area,

then:

$$kt = \frac{m'_t}{m'_c} \times \frac{S'_c}{S'_t}$$

This coefficient varies according to the apparatus used.

5.2.8.2. *Concentration of mercaptoacetic acid present in the sample*

If ‘t’ represents mercaptoacetic acid:

let:

k_t its response factor,

S_t = its peak area.

If ‘c’ represents methyl octanoate:

let:

m_c = its mass (in mill grams) in the mixture,
S_c = its peak area,
M = the mass (in milligrams) of the initial test portion,

then the % (m/m) mercaptoacetic acid present in the sample is:

$$\frac{m_c}{M} \times \frac{k_t \times S_t}{S_c} \times 100$$

6. REPEATABILITY[1]

For a mercaptoacetic acid content of 8 % (m/m), the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0,8 % (m/m).

(**1**) Norm ISO 5725.